

**PHYLOGENETIC AND BIOINFORMATIC
ANALYSIS OF GENES RELATED TO IMMUNITY
AND RETROVIRAL ELEMENTS IN *NASALIS*
LARVATUS GENOME**

ASWINI LEELA LOGANATHAN



PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

UMS

**THESIS SUBMITTED IN FULFILLMENT FOR THE
DEGREE OF MASTER OF SCIENCE**

**BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH**

2014

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS THESIS

JUDUL : **PHYLOGENETIC AND BIOINFORMATIC ANALYSIS OF GENES RELATED TO IMMUNITY AND RETROVIRAL ELEMENTS IN *NASALIS LARVATUS* GENOME**

IJAZAH. : **SARJANA SAINS**

Saya Aswini Leela Loganathan, Sesi Pengajian 2012-2014, mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis ini adalah hak milik Universiti Malaysia Sabah
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat Salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat Salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.

☐ SULIT

(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)

☐ TERHAD

(Mengandungi maklumat TERHAD yang ditentukan oleh organisasi/ badan di mana penyelidikan dijalankan)

☒ TIDAK TERHAD

Disahkan oleh,

NURULAIN BINTI ISMAIL

LIBRARIAN

UNIVERSITI MALAYSIA SABAH

(PERPUSTAKAWAN)

ASWINI LEELA LOGANATHAN

ALAMAT: No2, Jalan Anggerik 15
Anggerik Villa, Taman Kulai Utama,
81000, Kulai, Johor

Tarikh: 7 Disember 2014

(PROF. MADYA. DR. VIJAY KUMAR)

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

7th December 2014



Aswini Leela Loganathan

MZ1211011T



PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

UMS
UNIVERSITI MALAYSIA SABAH

CERTIFICATION

NAME : **ASWINI LEELA LOGANATHAN**

MATRIC NO. : **MZ1211011T**

TITLE : **PHYLOGENETIC AND BIOINFORMATIC ANALYSIS OF
GENES RELATED TO IMMUNITY AND RETROVIRAL
ELEMENTS IN *NASALIS LARVATUS* GENOME**

DEGREE : **MASTER OF SCIENCE IN BIOTECHNOLOGY (MOLECULAR
GENETIC)**

VIVA DATE : **5TH DECEMBER 2014**



DECLARED BY

1. SUPERVISOR COMMITTEE

Assoc. Prof. Dr. Vijay Kumar

Dr. Kenneth Francis Rodrigues

Dr. Christopher Voo Lok Yung

Signature

A handwritten signature in black ink, appearing to read "Vijay Kumar", written over a horizontal line.

Two handwritten signatures in black ink. The top signature appears to read "K. F. Rodrigues" and the bottom signature appears to read "Christopher Voo Lok Yung". Both are written over horizontal lines.

ACKNOWLEDGEMENTS

To the Almighty God who has granted me all these graces to fulfill this work and who supported me and blessed me by His power and His mercy in all my life. To Him I extend my heartfelt thanks. Many institutions and individuals were responsible for the crystallization of this humble work, whose associations and encouragement have contributed to the accomplishment of the present thesis, and I would like to pay tribute to all of them. I specially wish to express my sincere thanks and gratitude to my supervisor, Dr. Kenneth Francis Rodrigues for his kindness, admirable supervision, direct guidance, generous considerations and valuable support during my research under him. Not forgetting his fruitful discussions, constructive advises, and particularly for sharing his expertise in molecular genetics and revision of this thesis and giving me the opportunity to do my research in University Malaysia Sabah (UMS). I would also like to express my gratefulness to my co - supervisors of my thesis, Assoc. Prof. Dr. Vijay Kumar and Dr. Christopher Voo Lok Yung, for their motivations, and for teaching me very precious knowledge about molecular genetics and providing basic bioinformatic knowledge and offering me such a kind of opportunity regarding my research and their continuous encouragement and kind advises. My deepest thanks to all the staff members and colleagues of the Biotechnology Research Institute for their kindness and guidance and for always helping me with the research. My gratitude to University Malaysia Sabah (UMS) for giving me this opportunity to further increase my knowledge and experience by helping me to do my research in this University. Lastly I would like to extend my special thanks to my family and friends for their never ending love, support and encouragement.

ASWINI LEELA LOGANATHAN

7TH DECEMBER 2014

ABSTRACT

The Proboscis monkey (*Nasalis larvatus*) also known locally as *Monyet Belanda / Bayau* are endemic to the island of Borneo. Unfortunately the *N. larvatus* habitat loss has resulted in severe decline in endemic population and listed in Appendix I by the Convention on International Trade in Endangered Species (CITES). This research is divided into two parts. The first part of this research focused on the Mitochondrial DNA (mtDNA) of the *N. larvatus*. At present, there are only 2 data available for *N. larvatus* mtDNA and this research served as a benchmark for validating those data. Furthermore the importance of this study is to amplify the mitochondrial genome of the species *Nasalis larvatus* from two regions which is Sabah and Sarawak, which is then utilized for sequencing and ultimately comparative mitochondrial genomics with other Primates. Second part of this research focused on characterizing the genes linked to immunity and host defence which are the Major histocompatibility complex (MHC), Defensins and Retroviral elements present in the *N. larvatus* genome. In order to achieve this, the polymerase chain reaction (PCR) primers were designed from a total of 31 immune genes which were MHC, Defensins and Retroviruses of Non human primate genes obtained from EST. Cloning was carried out using CloneJET PCR cloning kit (Thermo Scientific). The plasmid was purified using Gene Jet Plasmid Mini Prep Kit (Thermo Scientific). The plasmid was then sequenced using BigDye Terminator v3.1 cycle sequencing kit chemistry. All novel immunology and retrovirus sequences have been deposited in GenBank (accession no: KF279631-KF279640, KP064501-KP064501 and KM889667). From the study, the mitochondrial genome of *N. larvatus* from Sabah and Sarawak is 16,574 bp and 16,573 bp long, respectively. Selection at the amino acid level was measured as the rates of nonsynonymous (d_N) and synonymous (d_S) substitutions per codon site. From the data, mtDNA genes underwent neutral selection. For the MHC data, there were no positive selection detected while for defensin and retrovirus data, it shows that some primates underwent neutral selection and some underwent positive selection. The phylogenetic tree was created using the Neighbor-Joining and maximum likelihood method. The MHC data shows that the tree topology is orthologous with the primates. As for the defensins, phylogenetic tree results show it is highly paralogous with *Macaca mulatta* while as for the Retroviruses the phylogenetic tree shows orthologous divergent. Because the two different methods used, give trees with the same topology, the phylogenetic relationships derived from these sequence comparisons appear reliable. In this study, the aim is not to get the complete coverage of the retroviral and immunology genes but to compare the sequences to *N. larvatus* to see if they are homologous.

ABSTRAK

ANALISIS FILOGENETIK DAN BIOINFOMATIK GENE GENE YANG BERKAITAN DENGAN IMUNITI DAN ELEMEN RETROVIRAL DI DALAM GENOM NASALIS LARVATUS.

Nasalis larvatus (Monyet Belanda atau Bayau) adalah endemik pada kepulauan Borneo. Malangnya *N. larvatus* ini disenaraikan sebagai lampiran I oleh Convention on International Trade in Endangered Species (CITES). Kajian ini dibahagikan kepada dua bahagian. Bahagian pertama kajian ini akan memberi tumpuan kepada DNA mitokondrium (mtDNA) daripada *N. larvatus*. Maklumat yang diperolehi akan membantu dalam mentafsir order filogenetik dalam Kingdom Animalia. Pada masa ini, hanya terdapat 2 data berkaitan mtDNA *N. larvatus* dan penyelidikan ini boleh dijadikan sebagai tanda aras bagi mengesahkan data tersebut. Bahagian kedua kajian ini akan menumpukan pada pengkarektoran gen yang dikaitkan dengan imuniti dan Major histocompatibility complex (MHC), Defensins dan unsur-unsur Retroviral yang hadir dalam genom *N. larvatus*. Gen-gen ini mempunyai beberapa peranan yang unik selain memainkan peranan penting dalam sistem pertahanan badan. Bagi mencapai matlamat ini, Rantaian tindak balas (PCR) polymerase direka dari sejumlah 31 gen imun yang terdiri dari MHC, Defensins dan retrovirus gen manusia dan primat yang diperolehi dari EST. Kloning dibuat menggunakan kit pengklonan CloneJET PCR (Thermo saintifik). Plasmid dipurifikasi dengan menggunakan kit Gene Jet Plasmid Mini Prep (Thermo saintifik). Kemudian, plasmid yang dipurifikasi itu disequenced menggunakan kit kimia BigDye Terminator v3.1 kitaran penjujukan. Kesemua sequence imunologi dan retrovirus novel ini telah dipamerkan di GenBank (Nombor akses: KF279631 - KF279640 , KP064501-KP064501 dan KM889667). Dari kajian ini , genom mitokondria *N. larvatus* dari Sabah dan Sarawak adalah 16574 dan 16573 bp masing-masing. Pemilihan di peringkat asid amino telah diukur sebagai kadar substitusi nonsynonymous (dN) dan synonymous (dS) pada setiap codon. Dari data itu, gen mtDNA telah menjalani pemilihan neutral. Untuk data MHC, tidak ada pilihan positif dikesan manakala bagi data defensin dan retrovirus, ia menunjukkan bahawa segelintir primat menjalani pemilihan neutral dan segelintir menjalani pemilihan positif. Pokok filogenetik telah dicipta menggunakan kaedah Neighbor-joining dan Maximum likelihood. Data menunjukkan bahawa, bagi data MHC, topologi pokok adalah ortologos dengan primat . Bagi data defensins, keputusan pokok filogenetik menunjukkan ia adalah paralogous dengan *Macaca mulatta* manakala untuk retrovirus pokok filogenetik menunjukkan ortologos dengan primat. Oleh kerana kedua-dua kaedah yang berbeza digunakan, memberikan pokok dengan topologi yang sama, hubungan filogenetik yang diperolehi daripada perbandingan urutan ini adalah dipercayai . Dalam kajian ini, matlamatnya bukan untuk mendapatkan liputan yang lengkap gen retroviral dan imunologi tetapi untuk membandingkannya dengan *N. larvatus* bagi melihat jika ianya adalah homolog.

TABLE OF CONTENT

	Page
TITLE	i
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENT	iv
ASBTRACT	v
<i>ABSTRAK</i>	vi
LIST OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xiii
LIST OF PHOTOGRAPHS	xvi
LIST OF ABBREVIATIONS	xvii
LIST OF APPENDICES	xviii

CHAPTER 1: INTRODUCTION

1.1	Background	1
1.2	Significant of study	2
1.3	Research Objective	4

CHAPTER 2: LITERATURE REVIEW

2.1	Primates and Old World Monkey	6
2.2	The Proboscis Monkey (<i>Nasalis larvatus</i>)	9
2.2.1	Morphology of the Male, Female and Juvenile Proboscis Monkey	10
2.2.2	Habitat of the Proboscis Monkey	12
2.2.3	Conservation Status of the Proboscis Monkey	13
2.3	Mitochondrial DNA (mtDNA)	
2.3.1	Mitochondrial DNA and Ancestral Information, Nucleotide Substitution Rate	18
2.3.2	mtDNA Loop (D-Loop)	19
2.3.3	Haplotypes/ Haplogroup Determination	21
2.4	The Immune System of the Primates	22

2.4.1	The Innate Immune System	23
2.4.2	The Adaptive Immune System	23
a.	Forms of Adaptive Immunity	24
b.	The Roles of MHC Molecules in Adaptive Immune Responses	24
2.4.3	Major Histocompatibility Complex (MHC)	25
a.	Role of MHC in Host Defence	27
b.	Role of MHC in Kin Recognition	28
c.	Role of MHC in Mate Choice	30
2.4.4	Antimicrobial Peptides	33
a.	Toll Like Receptors (TLR)	34
b.	Defensins	35
i.	Alpha Defensin	37
ii.	Beta Defensin	38
iii.	Theta Defensin	39
2.4.5	Other Immunity Genes (The Duffy Gene)	40
2.5	Mobile Element	41
2.5.1	Transposable Elements	42
2.5.2	Retroviruses	43
2.5.3	Endogenous Retrovirus	44
a.	Human Endogenous Retrovirus (HERV)	45
b.	Tripartite Motif (TRIM) Genes	46
2.6	Bioinformatic Analysis of the Proboscis Monkey and other Primates	47
2.6.1	Phylogenetic Analysis of the Proboscis Monkey and other Primates	47
2.6.2	Comparative Studies of the Proboscis Monkey and other Primates	48

CHAPTER 3: MATERIALS AND METHODS

3.1	Overview of Methodology	50
3.2	<i>In Vitro</i> Analysis	50
3.2.1	DNA Extraction of <i>N. larvatus</i> from Sabah	50
3.2.2	Primer Design	51

a.	Primer design of Roy's mtDNA	51
b.	Primer Design of <i>N. larvatus</i> Immunity and Retroviral Genes	53
3.2.3	Polymerase Chain Reaction (PCR) of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	58
3.2.4	Gel Extraction of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	59
3.2.5	Cloning of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	59
a.	DNA Ligation	60
b.	Transformation of DNA into competent <i>E. coli</i> cells	60
3.2.6	Colony PCR of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	60
3.2.7	Plasmid Mini Prep <i>N. larvatus</i> mtDNA, Immunity	61
3.2.8	Sequencing of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	61
3.3	<i>In Silico</i> Analysis	62
3.3.1	Sequence Assembly and Alignment of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	62
3.3.2	Similarity Analysis of mtDNA of <i>N. larvatus</i> of Roy (Sabah) and Charlie (Sarawak)	67
3.3.3	Comparative Analysis of mtDNA of <i>N. larvatus</i> of Roy (Sabah) and Charlie (Sarawak)	67
3.3.4	Maximum Likelihood Estimate of Gamma Parameter for Site Rates of <i>N. larvatus</i> mtDNA	68
3.3.5	Protein structure of <i>N. larvatus</i> Immunity and Retroviral Genes	68
3.3.6	Nucleotide Composition of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	68
3.3.7	Pairwise Distance of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	68
3.3.8	Positive Selection Test of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	69

3.3.9	Phylogenetic Reconstruction of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	69
3.3.10	Maximum Likelihood Estimate of Transition/Transversion Bias of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	70

CHAPTER 4: RESULTS

4.1	Overview of Results	71
4.2	<i>In Vitro</i> Analysis	72
4.2.1	DNA Extraction of <i>N. larvatus</i> from Sabah	72
4.2.2	Primer Design of Roy's mtDNA	72
4.2.3	Polymerase Chain Reaction (PCR) of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	73
4.2.4	Gel Extraction of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	76
4.2.5	Cloning of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	77
4.2.6	Colony PCR of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	79
4.2.7	Plasmid Mini Prep <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	79
4.2.8	Sequencing of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	80
4.3	<i>In Silico</i> Analysis	82
4.3.1	Sequence Assembly and Alignment of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	82
4.3.2	Similarity Analysis of mtDNA of <i>N. larvatus</i> from Sabah (Roy) and Sarawak (Charlie)	82
4.3.3	Comparative Analysis of mtDNA of <i>N. larvatus</i> from Sabah (Roy) and Sarawak (Charlie)	83
4.3.4	Maximum Likelihood Estimate of Gamma Parameter for Site Rates of <i>N. larvatus</i> mtDNA	85

4.3.5	Protein structure of <i>N. larvatus</i> Immunity and Retroviral Genes	85
4.3.6	Nucleotide Composition of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	85
4.3.7	Pairwise Distance of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	91
4.3.8	Positive Selection Test of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	101
4.3.9	Phylogenetic Analysis of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	112
4.3.10	Maximum Likelihood Estimate of Transition/Transversion Bias of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	125
 CHAPTER 5: DISCUSSION		
5.1	Overview of Discussion	127
5.2	<i>In Vitro</i> Analysis	129
5.2.1	DNA Extraction of <i>N. larvatus</i> from Sabah	130
5.2.2	Primer Design of Roy's mtDNA	130
5.2.3	Polymerase Chain Reaction (PCR) of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	131
5.2.4	Gel Extraction of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	131
5.2.5	Cloning of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	131
5.2.6	Colony PCR of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	132
5.2.7	Plasmid Mini Prep <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	132
5.2.8	Sequencing of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	133

5.3	<i>In Silico</i> Analysis	133
5.3.1	Sequence Assembly and Alignment of <i>N. larvatus</i> mtDNA, Immunity Genes and the Retroviral Genes	134
5.3.2	Similarity Analysis of mtDNA of <i>N. larvatus</i> from Sabah (Roy) and Sarawak (Charlie)	135
5.3.3	Comparative Analysis of mtDNA of <i>N. larvatus</i> from Sabah (Roy) and Sarawak (Charlie)	135
5.3.4	Maximum Likelihood Estimate of Gamma Parameter for Site Rates of <i>N. larvatus</i> mtDNA	135
5.3.5	Protein structure of <i>N. larvatus</i> Immunity and Retroviral Genes	136
5.3.6	Nucleotide Composition of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	137
5.3.7	Pairwise Distance of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	142
5.3.8	Positive Selection Test of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	147
5.3.9	Phylogenetic Analysis of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	153
5.3.10	Maximum Likelihood Estimate of Transition/Transversion Bias of <i>N. larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	162
CHAPTER 6: CONCLUSION AND RECOMMENDATION		167
REFERENCES		171
APPENDICES		203

LIST OF TABLES

	Page
Table 2.1:	6
Table 3.1:	51
Table 3.2:	54
Table 3.3:	55
Table 3.4:	56
Table 3.5:	57
Table 3.6:	58
Table 3.7:	64
Table 3.8:	64
Table 3.9:	65
Table 3.10:	66
Table 3.11:	66

Table 3.12:	Latin name, Common name and GeneBank accession numbers for primate taxa used in TRIM genes study	67
Table 4.1:	Results from the Tajima's test for <i>N. larvatus</i> mtDNA	83
Table 4.2:	Comparative Analysis of mtDNA from Sabah (Roy) and Sarawak (Charlie)	84
Table 4.3:	Nucleotide Composition of <i>N. larvatus</i> mtDNA and Other Primate's mtDNA	87
Table 4.4:	Nucleotide Composition of <i>N. larvatus</i> MHC and Other Primate's MHC	87
Table 4.5:	Nucleotide Composition of <i>N. larvatus</i> α -Defensin and Other Primate's α -Defensin	88
Table 4.6:	Nucleotide Composition of <i>N. larvatus</i> β -Defensin and Other Primate's β -Defensin	88
Table 4.7:	Nucleotide Composition of <i>N. larvatus</i> θ -Defensin and Other Primate's θ -Defensin	89
Table 4.8:	Nucleotide Composition of <i>N. larvatus</i> Retrovirus and Other Primate's Retrovirus	89
Table 4.9:	Nucleotide Composition of <i>N. larvatus</i> Duffy Genes and Other Primate's Duffy Genes	90
Table 4.10:	Nucleotide Composition of <i>N. larvatus</i> TRIM Genes and Other Primate's TRIM Genes	90
Table 4.11:	Pairwise Distance Estimates of Evolutionary Divergence between mtDNA Sequences	93
Table 4.12:	Pairwise Distance Estimates of Evolutionary Divergence between MHC Sequences	94
Table 4.13:	Pairwise Distance Estimates of Evolutionary Divergence between α -Defensin Sequences	95

Table 4.14:	Pairwise Distance Estimates of Evolutionary Divergence between β -Defensin Sequences	96
Table 4.15:	Pairwise Distance Estimates of Evolutionary Divergence between θ -Defensin Sequences	97
Table 4.16:	Pairwise Distance Estimates of Evolutionary Divergence between Retrovirus Sequences	98
Table 4.17:	Pairwise Distance Estimates of Evolutionary Divergence between Duffy Genes Sequences	99
Table 4.18:	Pairwise Distance Estimates of Evolutionary Divergence between TRIM Gene Sequences	100
Table 4.19:	Codon-based Test of Positive Selection for analysis between mtDNA sequences.	104
Table 4.20:	Codon-based Test of Positive Selection for analysis between MHC sequences	105
Table 4.21:	Codon-based Test of Positive Selection for analysis between α -Defensin sequences	106
Table 4.22:	Codon-based Test of Positive Selection for analysis between β -Defensin sequences	107
Table 4.23:	Codon-based Test of Positive Selection for analysis between θ -Defensin sequences	108
Table 4.24:	Codon-based Test of Positive Selection for analysis between Retrovirus sequences	109
Table 4.25:	Codon-based Test of Positive Selection for analysis between Duffy Genes sequences	110
Table 4.26:	Codon-based Test of Positive Selection for analysis between TRIM genes sequences	111
Table 4.27:	Maximum Likelihood Estimate of <i>N. larvatus</i> Transition/ Transversion of Immunology and Retrovirus Genes	126

Figure 4.9:	Example of Colony PCR of <i>N. larvatus</i> mtDNA Genes using CloneJET PCR cloning kit (Thermo Scientific)	79
Figure 4.10:	Example of Plasmid DNA of mtDNA isolated by the Plasmid miniprep technique.	80
Figure 4.11:	Example of an Electropherogram of <i>N. larvatus</i> mtDNA sequences	81
Figure 4.12:	Example of Alignment using ClustalW program implemented in MEGA5.2	82
Figure 4.13:	Maximum Likelihood Tree describing genetic similarity among the <i>N. larvatus</i> mtDNA and other primates' mtDNA	115
Figure 4.14:	Neighbor-Joining Tree describing genetic similarity among the <i>N. larvatus</i> mtDNA and other primates' mtDNA	116
Figure 4.15:	Maximum Likelihood Tree describing genetic similarity among the <i>N. larvatus</i> mtDNA and other primates' mtDNA	116
Figure 4.16:	Neighbor-Joining Tree describing genetic similarity among the <i>N. larvatus</i> MHC and other primates' MHC	117
Figure 4.17:	Maximum Likelihood Tree describing genetic similarity among the <i>N. larvatus</i> MHC and other primates' MHC	117
Figure 4.18:	Neighbor-Joining Tree describing genetic similarity among the <i>N. larvatus</i> α -Defensin and other primates' α -Defensin	118
Figure 4.19:	Maximum Likelihood Tree describing genetic similarity among the <i>N. larvatus</i> α -Defensin and other primates' α -Defensin	118
Figure 4.20:	Neighbor-Joining Tree describing genetic similarity among the <i>N. larvatus</i> β -Defensin and other primates' β -Defensin	119

Figure 4.21:	Maximum Likelihood Tree describing genetic similarity among the <i>N. larvatus</i> β -Defensin and other primates' β -Defensin	119
Figure 4.22:	Neighbor-Joining Tree describing genetic similarity among the <i>N. larvatus</i> θ -Defensin and other primates' θ -Defensin	120
Figure 4.23:	Maximum Likelihood Tree describing genetic similarity among the <i>N. larvatus</i> θ -Defensin and other primates' θ -Defensin	120
Figure 4.24:	Neighbor-Joining Tree describing genetic similarity among the <i>N. larvatus</i> Retrovirus genes and other primates' Retrovirus genes	121
Figure 4.25:	Maximum Likelihood Tree describing genetic similarity among the <i>N. larvatus</i> Retrovirus genes and other primates' Retrovirus genes	121
Figure 4.26:	Neighbor-Joining Tree describing genetic similarity among the <i>N. larvatus</i> Duffy genes and other primates' Duffy genes	122
Figure 4.27:	Maximum Likelihood Tree describing genetic similarity among the <i>N. larvatus</i> Duffy genes and other primates' Duffy genes	122
Figure 4.28:	Neighbor-Joining Tree describing genetic similarity among the <i>N. larvatus</i> TRIM genes and other primates' TRIM genes	123
Figure 4.29:	Maximum Likelihood Tree describing genetic similarity among the <i>N. larvatus</i> TRIM genes and other primates' TRIM genes	123
Figure 4.30:	Neighbor-Joining Tree describing <i>N. larvatus</i> and other Primates Immunology and Retroviral genes	124

LIST OF PHOTOGRAPHS

	Page
Photograph 2.1: A Male Proboscis Monkey from the Labuk Bay Proboscis Monkey Sanctuary, Sandakan	11
Photograph 2.2: Male and Female Proboscis Monkey feeding on fruits at the Labuk Bay Proboscis Monkey Sanctuary, Sandakan	13



UMS
UNIVERSITI MALAYSIA SABAH

LIST OF ABBREVIATIONS

mtDNA	-	Mitochondrial DNA
DNA	-	Deoxyribonucleic acid
MHC	-	Major Histocompatibility Complex
TLR	-	Toll Like Receptor
OWM	-	Old World Monkey
DARC	-	Duffy Antigen Receptor for Chemokines
ERV	-	Endogenous Retroviruses
HERV	-	Human Endogenous Retrovirus
TRIM	-	Tripartite Motif
NJ	-	Neighbour-Joining
ML	-	Maximum Likelihood
%	-	Percent
>	-	More than
<	-	Less Than
α	-	Alpha
β	-	Beta
θ	-	Theta
°C	-	Degree Celsius
BLAST	-	Basic alignment search tool
bp	-	Base pair
dNTP	-	Deoxynucleoside-5'-triphosphate
dH₂O	-	Distilled Water

EDTA	-	Ethylenediaminetetra-acetate
e-value	-	Expected value
mm	-	Milimeter
kb	-	Kilo base
mM	-	Milimolar
ml	-	Mililitre
MgCl₂	-	Magnesium Chloride
μ	-	Micro
M	-	Molar
μL	-	Microlitre
μg	-	Microgram
μmol	-	Micromole
nm	-	Nanometer
PCR	-	Polymerase Chain Reaction
RNA	-	Ribonucleic Acid
TBE	-	Tris-Boric Acid- EDTA
U	-	Unit
V	-	Volt
ti	-	Transition
tv	-	Transversion
dS	-	Synonymous
dN	-	Non-Synonymous



LIST OF APPENDICES

Appendix A	Sequencing Result of <i>Nasalis larvatus</i> mitochondrion DNA (Roy)	210
Appendix B	Sequencing Result of <i>Nasalis larvatus</i> Immunity Genes and Retroviral Genes	215
Appendix C	Protein Structure of <i>Nasalis larvatus</i> Immunity Genes and Retroviral Genes	222
Appendix D	Positive Selection of <i>Nasalis larvatus</i> Immunity Genes and Retroviral Genes	226
Appendix E	Test of the Homogeneity of Substitution Patterns between Sequences and Estimation of Net Base Composition Bias Disparity Between mtDNA Immunity Genes and Retroviral Genes Sequences	230
Appendix F	Comparison of Nucleotide Composition of <i>Nasalis larvatus</i> mtDNA, Immunity Genes and Retroviral Genes	236



UMS
UNIVERSITI MALAYSIA SABAH

CHAPTER 1

INTRODUCTION

1.1 Background

Sabah, (72,000km²) is a Malaysian state which occupies the north-eastern tip of Borneo Island in Southeast Asia. It is situated within one of the richest biogeographical regions equally in terms of species and habitat richness. Its non-human primate fauna in particular varies greatly. Thirteen species of non-human primate have been known to live on Borneo Island, and 10 species are reputable in Sabah. Amongst the most outstanding non-human primate species is the Bornean endemic Proboscis monkey (*Nasalis larvatus*). *Nasalis larvatus* is endemic to the island of Borneo and they favour coastal regions to inland areas.

The Proboscis monkey also known locally as *Monyet Belanda / Bayau* are widespread in Borneo and can be found in Brunei, Indonesia (Kalimantan) and Malaysia (Sabah and Sarawak). These Old World Monkeys which separated from the human lineage 25 million years ago are iconic to Sabah and serve as one of the major tourist attractions in Sabah. Despite being on earth for such a long period, habitat loss has led to the decline in endemic population and listed by the Convention on International Trade in Endangered Species (CITES) as Appendix I.

The alarming rate in the declination of the population had researchers light upon effective measure to control the situation. The rationale of this study is to first help find unique genes in the whole genome of the Proboscis Monkey that could contribute in the conservation of the species and secondly to find genes that could help researchers in deciphering the role and function of genes that might be involved in immunity. By knowing the role and function of these genes, it might provide clues on how the species adapt to certain diseases and also find out if these genes are conserved in the Proboscis monkey and in human as well.

1.2 Significant of Study

Maternally inherited mtDNA is proven to be valuable for investigating intraspecific variation, population structure, phylogeography, and demography in these species. Recent phylogenetic studies divulge that mtDNA sequences are able to offer sufficient resolution for reconstructing a strong phylogeny. These data produce a reliable phylogeny featuring robust support values and most conspicuously contribute to resolution of the phylogeny of *Nasalis* species.

Previous studies have reported genetic variation of Proboscis monkey; there is limited genetic information available regarding this species (Meijaard & Nijman, 2000a). To get an understanding of genetic variation and phylogeny of Proboscis monkey, the mitochondrial DNA of the species from the island of Borneo should be analysed. This species is almost never seen in captivity outside of Borneo, and it is quite rare in zoos as well. While previous studies have lent insights into the taxonomy and phylogeny of the genus, phylogenetic relationships estimates on Proboscis Monkey remain unclear. As such, this study is an attempt to understand the genetic basis adaptation of these critically endangered primates by constructing a highly resolved phylogenetic tree with strong support for relationship between Proboscis monkey and other primates using Maximum likelihood and Neighbour-joining trees.

The major histocompatibility complex (MHC) consists of the most diverse genes identified in vertebrates (Knapp, 2005). Many decades of genetic research revealed that MHC genes play a critical role in the immune response and disease resistance. According to more recent studies, it is suggested that MHC genes influence individual odours used in kin recognition and mate choice as the MHC polymorphism acts as a tool for discriminating among individuals of the same species. For these reasons, the genes of the MHC can provide important perception into evolutionary origins, natural selection, and behavioural ecology in humans and nonhuman primates (Setchell *et al.*, 2010; Zelano *et al.*, 2002). In addition to external fitness related traits, the signal used in MHC based mate choice may also depend on odour which allows distinguishing MHC identities. MHC complexes that are not sustained at the surface of the cell but alternatively released into the